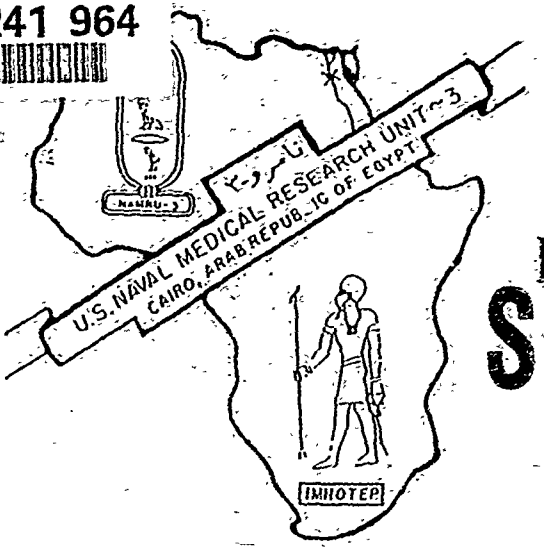
(2)



PUBLICATION REPORT

1628

110/89-90

RAPID DIAGNOSIS OF TUBERCULOUS MENINGITIS BY FREQUENCY-PULSED ELECTRON-CAPTURE GAS-LIQUID CHROMATOGRAPHY DETECTION OF CARBOXYLIC ACIDS IN CEREBROSPINAL FLUID

BY

J.B. Brooks, M.I. Daneshvar, R.L. Haberberger and I.A. Mikhail

U.S. NAVAL MEDICAL RESEARCH UNIT NO.3
(CAIRO, ARAB REPUBLIC OF EGYPT)
FPO NEW YORK 09527

JOURNAL OF CLINICAL MICROBIOLOGY, May 1990, p. 989–997
0095-1137/90/050989-09$02.00/0

Vol. 28, No. 5

Rapid Diagnosis of Tuberculous Meningitis by Frequency-Pulsed Electron-Capture Gas-Liquid Chromatography Detection of Carboxylic Acids in Cerebrospinal Fluid

J. B. BROOKS,[1]* M. I. DANESHVAR,[1] R. L. HABERBERGER,[2] AND I. A. MIKHAIL[2]

Analytical Chemistry Laboratory, Meningitis and Special Pathogens Branch, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333,[1] and Department of the Navy, U.S. Naval Medical Research Unit No. 3, Fleet Post Office, New York, New York 09527-1600[2]

Received 11 October 1989/Accepted 19 January 1990

The frequency-pulsed electron-capture gas-liquid chromatography technique described previously by Brooks et al. was modified and applied to the studies of coded and routine clinical specimens. Uncentrifuged cerebrospinal fluid (2 ml) was extracted under acidic conditions, derivatized, and analyzed by frequency-pulsed electron-capture gas-liquid chromatography on large-bore fused silica polar and nonpolar capillary columns. The frequency-pulsed electron-capture gas-liquid chromatography profile of carboxylic acids (C_2 through C_{32}) along with identification of tuberculostearic acid, established by retention time comparison of derivatized tuberculostearic acid and derivatized sample extract, strongly suggests the presence of *Mycobacterium tuberculosis* in patients with lymphocytic meningitis. Results from 41 coded cases and 75 clinical cases showed that the frequency-pulsed electron-capture gas-liquid chromatography test had a specificity of 91% and a sensitivity of 95%.

One of the major diagnostic problems remaining to be solved concerns infectious and noninfectious diseases that present a clinical picture of lymphocytic meningitis. Such diseases often are not readily diagnosed through clinical examination, and these types of infectious agents are difficult to isolate and culture. Many of these types of causative agents are never identified (9). Tuberculous meningitis, which is caused by *Mycobacterium tuberculosis*, is a lymphocytic type meningitis, and about a month is required to culture the organism. Even then, less than 50% of the positive cases are culture-positive (9). Attempts to develop rapid diagnostic tests based on conventional approaches have been ineffective, principally because of the extremely low number of organisms (4) present in the cerebrospinal fluid (CSF) at the time that the patient becomes ill.

Frequency-pulsed electron-capture gas-liquid chromatography (FPEC-GLC) has been used to detect low femtomole quantities of tuberculostearic acid (TSA) in CSF, serum, and pleural fluid (3, 4). FPEC-GLC has also been used to study carboxylic acid profiles of other acids in these fluids (5). These studies indicated that FPEC-GLC might be used to rapidly diagnose tuberculous meningitis within 3 h. The purpose of this investigation was to obtain additional information about the specificity and sensitivity of FPEC-GLC as a diagnostic test for tuberculous meningitis.

MATERIALS AND METHODS

Samples. The CSF specimens from U.S. Naval Research Unit no. 3 (NAMRU-3) were from clinical cases in Cairo, Egypt (Table 1). They were stored and shipped frozen; the volume of these samples ranged from 1 to 2 ml, and all of the specimens were centrifuged. The CSF samples from clinical cases (Table 2) were also stored and shipped frozen. They were 2 ml in volume and were not centrifuged. The NAMRU-3 samples were from culture-proven cases of meningitis, and several cases were of a nonlymphocytic type of meningitides. All specimens were free of blood, which, if present, will change the FPEC-GLC profile to contain peaks not normally seen in blood-free CSF. However, the detection of TSA is usually not affected by the presence of blood in the specimen. The NAMRU-3 specimens were used in two coded studies. The clinical cases from the United States and Canada were also analyzed and coded without knowing the final diagnosis until after the FPEC-GLC analysis. The following information was supplied with most cases: it was a lymphocytic type of meningitis, and there was no growth from a 24-h culture. Drug treatment and underlying disease were sometimes given. The samples were analyzed, and our FPEC-GLC test results along with a set of forms were returned to the physician with a request for final evaluation of the case and any additional clinical information. There was an 85% reply. In cases where there was no reply the FPEC-GLC data were not included. Table 2 contains the data from clinical cases.

Sample preparation. A 0.1-ml of 50% (vol/vol) H_2SO_4 along with 0.1 ml of an internal standard solution containing 7.6 pmol of heptanoic acid (C_7) in distilled H_2O was placed in a 50-ml round-bottom centrifuge tube. The tube was then taken to a biological safety cabinet, the uncentrifuged specimen was mixed gently, 2 ml of CSF or serum was added to the tube, and the contents were mixed by gentle shaking by hand. The sample was checked with pH paper to ensure that it was acidic (pH 2) and allowed to sit at room temperature for 2 min. Then, 20 ml of nanograde chloroform ($CHCl_3$, Mallinckrodt Scientific Products, Stone Mountain, Ga.) was added, and the tube was sealed with a Teflon-lined screw cap to avoid contamination from rubber-lined caps. Next, the sample was shaken for 5 min with a Burrell wrist-action shaker at a setting of 10. The $CHCl_3$ layer was then decanted into a 20- by-150-mm screw-cap test tube and evaporated in a fume hood to 1.5 ml with clean, dry $CaSO_4$-filtered air at 100°C in an analytical evaporator (Meyer, Northborough, Mass.) filled with sand. The concentrated sample was transferred to a 3-ml conical tube with care to avoid any traces of

* Corresponding author.

TABLE 1. FPEC-GLC results from study of CSF[a] received from NAMRU-3, Cairo, Egypt

CDC[b] no.	Drug therapy	FPEC-GLC profile[c]	TSA	*M. tuberculosis* isolated	Other organisms isolated
CA2799	Unknown	+	−	−	*Neisseria meningitidis*, serogroup A
CA2805	−	−	−	−	None
CA2807	Unknown	+	+	−	*N. meningitidis*, serogroup A
CA2809	Unknown	−	−	−	*Haemophilus influenzae*
CA2810[d]	+	+	−	+	None
CA2812[e]	−	+	−	−	None
CA3005	Unknown	−	−	−	Bacterial meningitis
2 Specimens[f]	Unknown	−	−	−	*N. meningitidis*, serogroup B
3 Specimens[f]	Unknown	−	−	−	*Streptococcus pneumoniae*
18 Specimens[f]	−	−	−	−	None
8 Specimens[f]	Unknown	−	−	−	None
15 Specimens[f]	Unknown	+	+	+	None
2 Specimens[f]	−	+	−	+	None

[a] Samples of 1 ml of centrifuged CSF were used. Best results were obtained with 2 ml of uncentrifuged CSF.
[b] CDC, Centers for Disease Control.
[c] An FPEC-GLC profile of carboxylic acid is composed of all peaks (C_2 through C_{22}) detected in an analysis.
[d] Therapy can lower the detection limit of TSA.
[e] The pathologist of tuberculous meningitis was not excluded.
[f] Specimens were not coded.

water. The sample was then further concentrated in the fume hood to about 25 μl.

Preparation of TCE esters. A 1:12 dilution of trichloroethanol (TCE) (Eastman Kodak Co., Rochester, N.Y.) was prepared by adding 0.1 ml of TCE to 1.1 ml of xylene (this solution can be stored in the refrigerator for future use). Next, 10 μl of TCE reagent and 20 μl of heptafluorobutyric anhydride were added to the concentrated sample extract (25 μl) in the 3-ml conical tube. The sample was mixed by gentle shaking by hand, corked, and allowed to sit at room temperature for 32 min. An emulsion, which formed after the addition of TCE-heptafluorobutyric anhydride during the derivatization step, later cleared with the addition of 0.2 ml of $CHCl_3$ and 0.1 ml of xylene with gentle mixing. The clear solution was then evaporated to 10 μl with clean, dry air at 60°C in a sand bath. Extra care was taken to avoid evaporating the sample to complete dryness. Next, 0.4 ml of nanograde hexane (Mallinckrodt) was added to the sample.

Reversed-phase chromatography column cleanup. A disposable octadecyl (C_{18}) reversed-phase chromatography column (Analytichem International, Harbor City, Calif.) was placed in a VAC-ELUT (Analytichem) vacuum manifold, and 2 column volumes of methanol followed by 2 column volumes of hexane were aspirated through the column. Hexane was stopped at the top of the sorbent bed, the sample was added to the column and aspirated through the column at about 2 mm Hg (266.6 Pa) vacuum for about 2 min, and the eluent was discarded. The previously used 3-ml conical tube was washed twice with 1-ml volumes of diethyl ether (Fisher Scientific Co., Fair Lawn, N.J.) and placed in a rack to dry. Next, the TCE esters were eluted from the column into a 10- by 75-mm disposable test tube with 3 ml of hexane. The eluent was evaporated to about 2 ml with clean, dry air in a 60°C sand bath, 0.1 ml of xylene was added, and then the sample was transferred to the previously ether-washed 3-ml conical tube and further evaporated to 10 μl. Extra care was taken to avoid taking the sample to complete dryness. Then 100 μl of a 50% (vol/vol) xylene-ethanol solution was added to the sample as a final solvent. The solution was mixed by gentle shaking by hand, and the sample was transferred to a 0.3-ml Teflon-lined screw-cap glass inserted vial (Universal Scientific, Inc., Atlanta, Ga.). 1 μl of the sample was used for FPEC-GLC analysis.

FPEC-GLC analysis. TCE of carboxylic acids were analyzed on Perkin Elmer 3920B gas chromatographs fitted with dual-electron-capture 10-mCi ^{63}Ni detectors. One instrument was equipped with a nonpolar, large-bore, bonded-phase, fused-silica capillary column (0.53-mm inner diameter, 25-m length) that was coated with a 4.4-μm-thick film of OV-1 (Southern Lab Apparatus, North Augusta, S.C.), and a moderately polar, large-bore, bonded-phase, fused-silica capillary column (0.5-mm inner diameter, 30-m length) coated with a 1.0-μm-thick film of OV-1701 (J & W Scientific, Folsom, Calif.). A polar, large-bore, bonded-phase, fused-silica capillary column (0.32-mm inner diameter, 25-m length) coated with a 0.25-μm-thick film of OV-225 (Southern Lab Apparatus) was used in a second instrument. The instruments were fitted with 0.25-in. (ca. 0.625-cm) injector ports and connected to the capillary columns with 0.25-in. by 0.0625-in. (ca. 0.0625-cm) graphite-reducing ferrules. Helium was the carrier gas with a flow rate of 3 ml/min through the OV-1701 and OV-225 columns at 125°C and 4 ml/min through the OV-1 column. The instruments were equipped with flow controllers with a range from 0 to 10 lb/in^2. The OV-1 and OV-1701 columns were held isothermal at 90°C for 4 min and then programmed to 275°C at a rate of 4°C/min and held isothermal for 32 min. The OV-225 column was held isothermal at 90°C for 4 min and then programmed to 220°C at 2°C/min and held isothermal for 12 min. The sensitivity of the instruments was established by adjusting the make-up gas (95% argon–5% methane), the standing current from a range of 3.5 to 1, and the attenuation at either 512 or 256 so that the internal standard (C_7) gave a twice-full-scale peak with a 1-μl injection. New ^{63}Ni foils require higher standing current (3 to 3.5), less attenuation (256), and less make-up gas flow (60 to 65 ml/min). On the other hand, older ^{63}Ni foils used 1 month or more generally require a lower standing current (1 to 2), increased attenuation (512), and increased make-up gas flow (75 to 80 ml/min).

RESULTS

Figure 1 shows analyses of TCE esters of a derivatized standard mixture of carboxylic acids (C_2 to C_{22}), iso acids (iC_4, iC_5, and iC_6), unsaturated acids ($C_{14:1}$, $C_{16:1}$, $C_{18:1}$, and $C_{20:4}$), phenylacetic acid (PAA), and TSA. Aromatic and

TABLE 2. FPEC-GLC results from analysis of clinical specimens received from the United States and Canada[a]

CDC[b] no.[c]	Drug therapy	FPEC GLC profile[d]	TSA	Physician's evaluation	
				Tuberculous meningitis	Other disease
1 to 27	+	+	+	+	None
28 to 37	−	−	−	−	None
38[e]	Unknown	−	−	−	Granuloma
39[e]	−	−	−	−	Fibrinoid necrosis vaginitis
40	−	−	−	−	Myeloblastic leukemia
41[e]	−	−	−	−	None
42[e]	−	−	−	−	None
43	−	−	−	−	Arachnoiditis
44[e]	−	−	+	+	None
45	−	−	−	−	Lymphocytic meningitis
46	−	−	−	−	Glioblastoma of the brain
47	−	+	+	?	Chronic meningitis
48	+	+	−	−	Meningoencephalitis
49[e]	−	+	−	+	None
50	−	−	−	−	Transverse myelitis
51	+	−	−	−	Meningitis
52	+	−	−	−	Viral meningitis
53	−	−	−	−	Behcet's syndrome
54	−	−	−	−	Brucellosis
55[e]	+	+	−	+	None
56	−	−	−	−	Cryptococcal meningitis
57	−	+	−	−	Guillain-Barre syndrome
58	−		−	−	Encephalitis
59	Unknown	−	−	−	Chronic meningitis
60	Unknown	−	−	−	Viral encephalitis
61	+	−	−	−	None
62	Unknown	−	−	−	Altered mental state
63	−	−	−	−	Carcinomatous meningitis
64	−	−	−	−	Sarcoidosis
65	−	−	−	−	Neoplasm
66	+	−	−	−	AIDS with secondary infection
67	−	−	+	?	Probable viral meningitis
68	−	−	−	−	Histoplasmosis
69	−	+	+	?	Chronic meningitis
70	+	−	−	−	Viral meningitis
71[e]	−	−	−	−	None
72	−	−	−	?	Chronic meningitis
73[e]	−	+	−	−	None
74[f]	Unknown	−	+	−	*M. tuberculosis*
75	+	−	−	−	Cryptococcal meningitis

[a] All of the patients listed had clinical symptoms of tuberculous meningitis.
[b] CDC, Centers for Disease Control. Samples are numbered in sequence for convenience.
[c] An FPEC-GLC profile of carboxylic acids is composed of all peaks (C_2 through C_{22}) detected in an analysis.
[d] The reason for suspecting tuberculous meningitis is unknown.
[e] Therapy can lower the detection limit of TSA.
[f] We have considered this case as a false-positive, but it is debatable.

unsaturated acids shifted retention time relationships (eluting later than straight-chain acids of the same carbon chain length) on the polar column and to a lesser degree on the moderately polar column (Fig. 1B and C), compared with retention time relationships on the nonpolar column (Fig. 1A). Analysis on the highly polar OV-225 column (Fig. 1C) and the nonpolar OV-1 columns (Fig. 1A) made the best combination for identification, and TSA was better resolved on the OV-1 column; however, the OV-225 column cannot withstand the high temperature used with the OV-1 and OV-1701 columns. Therefore, the OV-225 column was used in a separate instrument, and the OV-1 and OV-1701 columns were used together in the same gas chromatograph oven.

Figure 2 shows chromatograms of TCE-derivatized acidic $CHCl_3$ extracts of both CSF and serum from patients positive for tuberculous meningitis along with normal healthy controls. Figure 2A shows the type FPEC-GLC profile detected from the CSF of a patient positive for tuberculous meningitis with no indication of a secondary disease. Observe that C_2, C_3, iC_4, C_4, iC_5, C_5, C_6, C_8, PAA, C_{10}, C_{12}, C_{14}, C_{15}, C_{16}, C_{17}, $C_{18:1}$, C_{18}, TSA, and unidentified peaks labeled C and E were present; however, with the exception of the internal standard, C_{16}, and C_{18}, other peaks were low in concentration. Figure 2B shows the FPEC-GLC profile obtained from analysis of 2 ml of normal CSF obtained from an individual submitting for a myelogram. With the exceptions of the internal standard and residual reagents (data not shown), the chromatogram in Fig. 2B is practically devoid of peaks. Figures 2C and D show the data obtained from FPEC-GLC analysis of sera taken from a patient with tuberculous meningitis and a normal control, respectively. The major differences in the control serum and the serum from the patient with tuberculous meningitis were increased

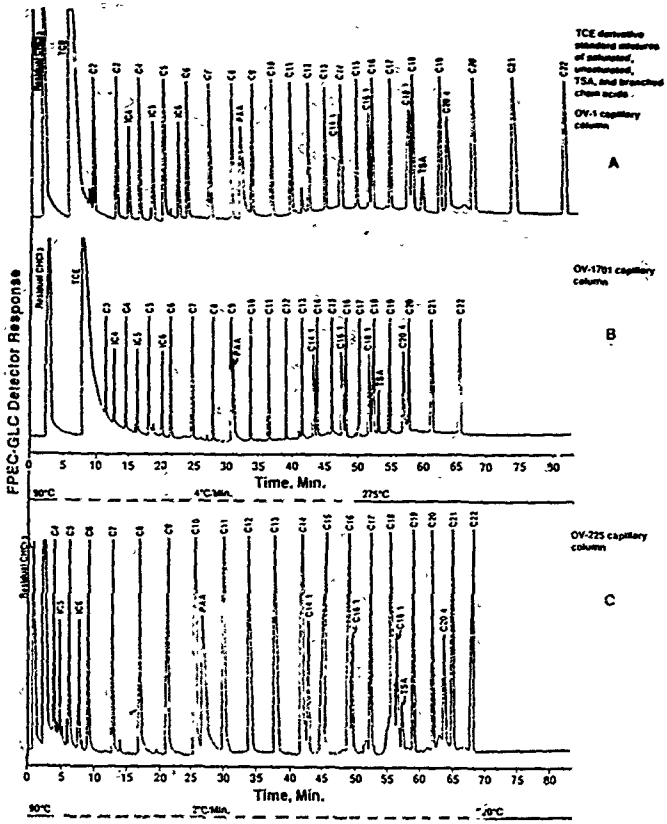


FIG. 1. FPEC-GLC chromatograms of TCE esters of a standard acid mixture (instructions for preparing standards for FPEC-GLC analysis are available upon request). The letter "C" followed by a number indicates a saturated carboxylic acid with the number of carbon atoms. The letter "i" is iso, and a colon between two numbers indicates unsaturation. The temperature program and analysis time are shown under chromatograms.

amounts of C_2, C_3, iC_4, C_4, unidentified peaks Un2, Un4, E, and Un6, and TSA. Some peaks were present in the control profile that were missing from the patient profile, and peaks C_{17} and $C_{20:4}$ were also reduced.

Figure 3 illustrates the process of computer scale expansion and determination of relative retention time for TSA based on comparison to C_{16} and C_{18}, spiking of the sample with standard TSA, and analyses on nonpolar and polar capillary columns. Figures 3A and C illustrate the measuring process to obtain relative retention times. The relative distances between C_{16}, C_{18}, and TSA is measured to 0.1 cm. This distance can change with age of the column. A standard mixture (Fig. 1) was analyzed every 3 weeks to verify column stability. The carboxylic acids C_7 (internal standard), C_{16}, and C_{18} were always present in both CSF and serum from patients with disease, and daily observations of retention time relationships of these acids were made to detect any possible retention time changes. As the column ages, usually after about a year, C_{16} and C_{18} will elute faster and closer together. Possible change can be detected by measurement of the distance between the retention times of C_{16} and C_{18} to 0.1 cm. In difficult cases where uncertainty existed concerning the identification of a peak, spiking the sample with about 23 fmol of derivatized standard TSA helped in the identification process (Fig. 3B and D). Chromatograms from the strip chart recorder can also be used to identify TSA by overlay of two chromatograms and by visual comparison to reference standards. For example, when chromatograms of TCE derivatives (Fig. 1) containing C_{16}, C_{18}, and TSA standards were aligned with the same acids on

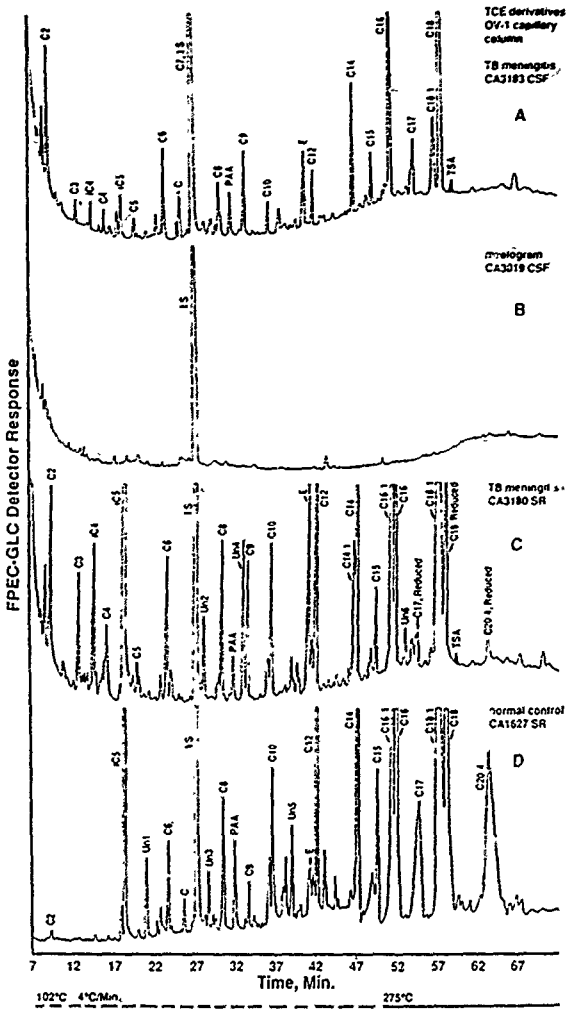


FIG. 2. FPEC-GLC chromatograms of TCE esters of CSF and serum (SR) samples from acidic $CHCl_3$ extracts. The type of bodily fluid and the disease are indicated on top right-hand corner of each chromatogram. TB, Tuberculous; Un, unknown. Peaks C and E indicate unknown compounds.

chromatograms obtained by FPEC-GLC analysis of CSF and or serum taken from different patients (Fig. 2A and C), a perfect overlay was obtained between C_{16}, C_{18}, and TSA.

Figure 4 shows data obtained by FPEC-GLC analysis of TCE-derivatized acidic $CHCl_3$ extract of CSF from a second patient (CA3117) positive for tuberculous meningitis (Fig. 4A) and chromatograms from three different patients suspected of having tuberculous meningitis (Fig. 4B, C, and D). The CSF from patient CA3051 was analyzed by FPEC-GLC (Fig. 4B) and reported to be negative for tuberculous meningitis; this patient was later determined to have carcinomatous meningitis. We have observed 52 cancer patients (data will be presented elsewhere) with this type of FPEC-GLC profile. The FPEC-GLC profile of the patient (CA3057) in

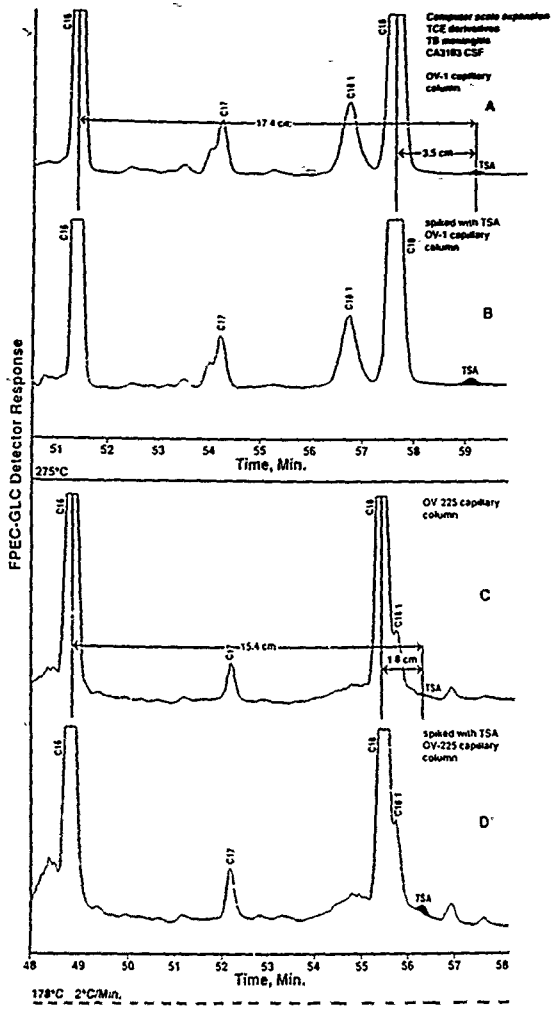


FIG. 3 Computer-expanded portions of FPEC-GLC chromatograms of a TCE-derivatized acidic $CHCl_3$ extract of CSF from a patient (CA3183) positive for tuberculous meningitis. Identification of TSA (the blackened peaks in chromatograms A and C) was made by determining relative retention times based on measurement of the distance (in centimeters) from the C_{16} and C_{18} peaks to the TSA peak and analyzing the TSA-spiked sample (chromatograms B and D) on two types of columns (polar OV-225 and nonpolar OV-1). The distance between peaks will change as the column ages; therefore, the relationship of eluting peaks must be checked about every 2 weeks by analysis of standards.

chromatogram C was obtained by analysis of CSF taken from a patient with acquired immunodeficiency syndrome (AIDS; CDC classification group IV), who was suspected of having tuberculous meningitis but was later determined to be negative for tuberculous meningitis by FPEC-GLC and other criteria. The FPEC-GLC profiles from the CSF of the terminally ill AIDS patient (Fig. 4C) and the cancer patient (Fig. 4B) were similar. We have observed this type of

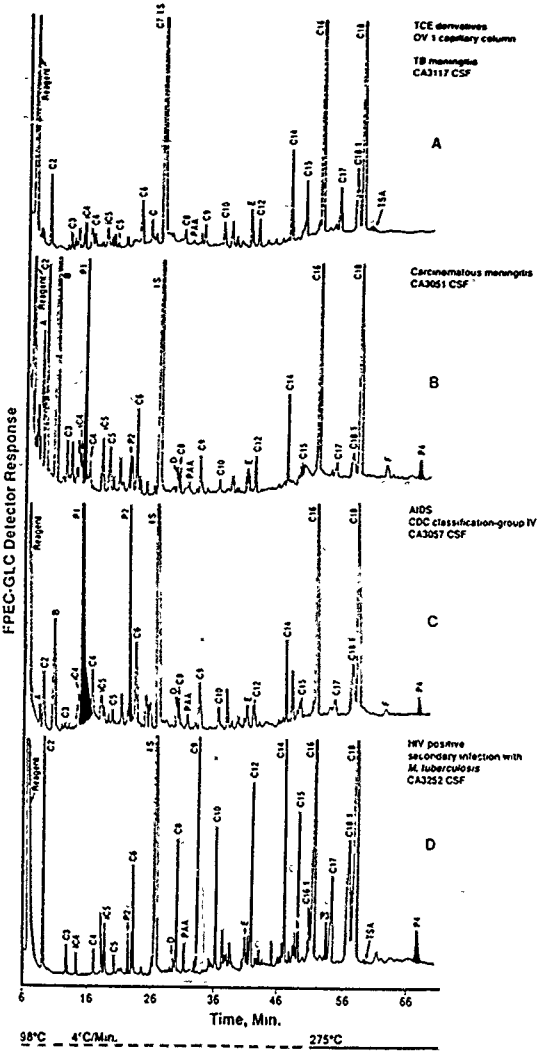


FIG 4 FPEC-GLC chromatograms of TCE esters of acidic $CHCl_3$ extracts of CSF Chromatogram A shows CSF a patient with tuberculous meningitis Chromatogram B is from a patient who was suspected of having tuberculous meningitis but was later determined to have carcinomatous meningitis. Chromatogram C is from a terminally ill patient with AIDS who was negative for tuberculous meningitis, and chromatogram D is from a patient who was positive for human immunodeficiency virus and who had tuberculous meningitis.

FPEC-GLC profile from 35 terminally ill patients with AIDS examined thus far (unpublished data). The FPEC-GLC profile of patient CA3252 (Fig. 4D) was obtained by analysis of CSF from a human immunodeficiency virus antibody-positive patient (the stage of the disease was not given, but AIDS had not developed). This patient was found to be positive for tuberculous meningitis by culture, and TSA was found (blackened peak at about 60 min) in the CSF by FPEC-GLC analysis. At this time we have analyzed the CSF and sera from four patients with AIDS; the samples were positive for *Mycobacterium avium*, but TSA was not detected. Figure 4D also illustrates a case of both primary (human immunodeficiency virus antibody positive) and secondary disease (*M. tuberculosis* infection) involvement. The presence of a full-scale peak of nononoic acid (C_9) along with unidentified peaks labeled P3 and P4 indicate an abnormal FPEC-GLC profile that would not be typical of cases positive for tuberculous meningitis alone (Fig. 4A), but the detection of TSA in the CSF of this patient along with the absence of TSA in *M. avium* patients strongly suggested infection with *M. tuberculosis*; this assumption was later confirmed by culture. The serum of this patient also contained TSA.

Table 1 presents data obtained from a collaborative research study with NAMRU-3 in Cairo, Egypt. Two coded studies and one noncoded study were conducted. The noncoded specimens are indicated in Table 1. Most of the tuberculous meningitis specimens analyzed as part of the NAMRU-3 study were culture positive. Some patients had begun therapy (Table 1). Data obtained from follow-up analysis of positive cases of tuberculous meningitis indicates that effective therapy for 5 to 7 days can lower the concentration of TSA in the CSF below the FPEC-GLC detection limits. One patient (Table 1, CA2812) had all of the clinical symptoms of tuberculous meningitis and an FPEC-GLC profile positive for tuberculous meningitis, including TSA, and improved with therapy, therefore, based on these data and reports by others (9) that about 50% of all positive cases are never cultured, we considered this case positive for tuberculous meningitis even though an organism was not recovered by culture. In the case (CA2807 in Table 1) that was false-positive, the FPEC-GLC profile, excluding TSA, was negative for tuberculous meningitis, and, because of a slight column change in elution characteristics, we had mislabeled a peak as TSA that had a retention time very close to that of TSA. Later when we began to determine the relative retention time for TSA (based on measurements from C_{16} and C_{18}), we recognized the reason for the misidentification.

Table 2 shows data obtained from the FPEC-GLC analysis of clinical specimens. The actual Centers for Disease Control numbers have been changed for purposes of clarity of the table. In cases 1 through 27, the clinical symptoms were positive, the FPEC-GLC profile was positive, TSA was positive, and the final evaluations of the physicians were positive based on response to drug therapy and/or culture. In cases 28 through 37 tuberculous meningitis was initially suspected, but FPEC-GLC results were negative, and the final evaluations of the physicians were negative with no indication of the causative agent. Throughout the remainder of Table 2, we show results from several pretreated cases and several cases from which we received a final evaluation from the attending physician. The cases that were submitted after therapy and had a positive FPEC-GLC profile but were negative for TSA were not considered false-negatives, since the test specified (as previously determined by research) the use of 2 ml of uncentrifuged CSF drawn before drug therapy. In one case (no. 74, which we included in our calculations as false-positive), tuberculosis was given as the final diagnosis. Since the patient was initially listed as having meningitis and was TSA positive, we now believe this was another case where someone failed to isolate *M. tuberculosis* from the CSF. We were not supplied with information on drug therapy administered to this patient before drawing the CSF sample.

Based on results listed in Tables 1 and 2, we calculated the specificity of the FPEC-GLC test for tuberculous meningitis to be 91% and the sensitivity to be 95%.

DISCUSSION

The results from this study show that FPEC-GLC can be used to rapidly diagnose tuberculous meningitis. Conventional approaches to rapid diagnosis of tuberculous meningitis have not been successful. Possible reasons for the lack of success are as follows: (i) the small number of the organisms that are present in the CSF, (ii) the host of diseases, infectious and noninfectious, that present a clinical picture similar to that of tuberculous meningitis (9), and (iii) the causative agents are difficult to isolate and diagnose. The diagnostic problem also adds importance to the FPEC-GLC test. Neoplasms are frequently involved in producing diagnostic problems because of the clinical pictures are similar to those found in cases of tuberculous meningitis (Table 2). Another area where FPEC-GLC is potentially beneficial involves the presence of secondary diseases such as in a patient with AIDS and tuberculous meningitis or patients with cancer and tuberculous meningitis. For TSA to be detected by FPEC-GLC analysis it must be nonbound (possess a free carboxyl group). At this time we have only detected TSA in the CSF and sera of patients positive for *M. tuberculosis*. In limited studies we have not detected nonbound TSA in positive cases of *M. avium* or *Nocardia* infection (5). In such cases the detection of TSA in the CSF by FPEC-GLC indicates infection with *M. tuberculosis*. Based on evidence presented in past reports (1–8) and data in this report, FPEC-GLC may eventually be useful as an aid to the diagnosis of some other type of diseases that give a clinical picture similar to that of tuberculous meningitis.

Other workers (10; P. A. Mardh, L. Larrson, N. Hibr H. C. Englebrack, and G. Odham, Letter, Lancet i:36 1983; G. L. French, C. Y. Chan, S. W. Cheung, R. Yeoh, M I. Humphries, and G. O. Mahony, Letter, Lancet i:855 1987) have reported the use of gas chromatography-mass spectrometry to detect TSA in the CSF of patients with tuberculous meningitis. We reported (4) the detection of TSA by gas chromatography-mass spectrometry in the CSF of patients with tuberculous meningitis, however, the concentration of TSA had to exceed 100 fmol per 2 ml for detection. Further, we found that in most positive cases the concentration of TSA was about 25 fmol per 2 ml of CSF. Although FPEC-GLC is capable of detecting 25 fmol of TCE-derivatized TSA per 2 ml of CSF, certain aspects of the test should be reemphasized. (i) The entire FPEC-GLC fatty acid profile (Fig. 2 and 4A, 7 to 70 min) along with TSA should be observed for determination of tuberculous meningitis. The fatty acids are low in quantity and number when compared with those in many other types of diseases so TSA must be identified by carefully measured retention time comparisons to 0.1 cm between known and unknown peaks obtained from analysis on high-resolution polar and nonpolar capillary columns or by overlay of a strip chart chromatogram with a chromatogram of derivatized standards obtained

from both polar and nonpolar column analysis. (iii) The internal standard (C_2) must be included before extraction with organic solvent, and the sensitivity of the instrument should be established on the basis of instrument response to the internal standard. (iv) Samples of 2 ml of noncentrifuged blood-free CSF preserved by freezing, with no added preservatives, should be used. (v) High-purity solvents, clean containers, and Teflon-lined screw-cap test tubes must be used for extraction and derivatization.

A sample can be derivatized and analyzed in about 3 h. Six derivatives can be prepared simultaneously, and each can be analyzed by FPEC-GLC in 70 min. Samples can be injected into the gas chromatograph with an autosampler, which permits analysis during nonworking hours, thus providing maximum use of the gas chromatograph. Additional benefits of automatic injections are more reproducible retention times with increased accuracy and precision. This is especially true where multiple operators are involved or the operator does not fully understand conditions than can cause variation.

LITERATURE CITED

1. Brooks, J. B., M. T. Basta, A. M. El Kholy, and C. W. Moss. 1984. Rapid differentiation of enterotoxigenic *Escherichia coli* that produce heat-stable and heat-labile toxins by frequency-pulsed electron-capture gas-liquid chromatography analysis of diarrheal stool specimen. J. Clin. Microbiol. 20:1145–1153.
2. Brooks, J. B., W. B. Cherry, L. Thacker, and C. C. Alley. 1972. Analysis by gas chromatography of amines and nitrosamines produced in vivo and in vitro by *Proteus mirabilis*. J. Infect. Dis. 126:143–153.
3. Brooks, J. B., R. B. Craven, D. Schlossberg, C. C. Alley, and F. M. Pritts. 1978. Possible use of frequency-pulse-modulated electron capture gas-liquid chromatography to identify septic and aseptic causes of pleural effusions. J. Clin. Microbiol. 8:203–208.
4. Brooks, J. B., M. I. Daneshvar, D. M. Fast, and R. C. Good. 1987. Selective procedures for detecting femtomole quantities of tuberculostearic acid in serum and cerebrospinal fluid by frequency-pulsed electron-capture gas-liquid chromatography. J. Clin. Microbiol. 25:1201–1206.
5. Brooks, J. B., J. V. Kasin, D. M. Fast, and M. I. Daneshvar. 1987. Detection of metabolites by frequency-pulsed electron-capture gas-liquid chromatography in serum and cerebrospinal fluid of a patient with *Nocardia* infection. J. Clin. Microbiol. 25:445–448.
6. Brooks, J. B., D. S. Kellogg, Jr., M. E. Shepherd, and C. C. Alley. 1980. Rapid differentiation of the major causative agents of bacterial meningitis by use of frequency-pulsed electron-capture gas-liquid chromatography: analysis of acids. J. Clin. Microbiol. 11:45–51.
7. Brooks, J. B., J. E. McDade, and C. C. Alley. 1981. Rapid differentiation of Rocky Mountain spotted fever from chicken pox, measles, and enterovirus infections and bacterial meningitis by frequency-pulsed electron-capture gas-liquid chromatography. J. Clin. Microbiol. 14:165–172.
8. Brooks, J. B., O. Nunez-Montiel, B. J. Wycoff, and J. C. Hierholzer. 1984. Studies of stools from pseudomembranous colitis, rotaviral, and other diarrheal syndromes by frequency-pulsed electron-capture gas-liquid chromatography. J. Clin. Microbiol. 20:549–560.
9. Eliner, J. J., and J. E. Bennett. 1976. Chronic meningitis. p. 350–351. The Williams and Wilkins Co., Baltimore.
10. French, C., Y. Chan, S. W. Cheung, and K. T. Oo. 1987. Diagnosis of pulmonary tuberculosis by detection of tuberculostearic acid in sputum by using gas chromatography-mass spectrometry with selected ion monitoring. J. Infect. Dis. 156:356–362.

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; Distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) 110/89-90	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION U.S. Naval Medical Research Unit No. 3	6b OFFICE SYMBOL (If applicable) NAVMEDRSCHU THREE
6c. ADDRESS (City, State, and ZIP Code) PSC 452, Box 5000 FPO, AE 09835-0007	7a NAME OF MONITORING ORGANIZATION 7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command	8b. OFFICE SYMBOL (If applicable) NAVMEDRSCH DEVCOM
8c. ADDRESS (City, State, and ZIP Code) National Naval Medical Center Building 1, Tower 12 Bethesda, MD 20889-5044	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

10 SOURCE OF FUNDING NUMBERS			
PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO	WORK UNIT ACCESSION NO.
62770A	3M1627-70A870	AQ	

11. TITLE (Include Security Classification)
Rapid Diagnosis of Tuberculous Meningitis by Frequency-Pulsed Electron-Capture Gas-Liquid Chromatography Detection of Carboxylic Acids in Cerebrospinal Fluid. (UNCLASSIFIED).

12 PERSONAL AUTHOR(S) Brooks, J.B.*, Daneshvar, M.I.*, Haberberger, R.L. and Mikhail, I.A.
* Bacterial Diseases Division, Center for Infectious Diseases, Centers for Diseases Control

13a. TYPE OF REPORT	13b. TIME COVERED FROM ____ TO ____	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
		1989, October, 11	9

16. SUPPLEMENTARY NOTATION
Published in: J. Clin. Microbiol., 28(5):989-997, 1990; Acc. No. 1628.

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Diagnosis; Chromatography; Lymphocytic meningitis; Cerebrospinal fluid; Mycobacterium tuberculosis.

19 ABSTRACT (Continue on reverse if necessary and identify by block number)
The frequency-pulsed electron-capture gas-liquid chromatography technique described previously by Brooks et al,. was modified and applied to the studies of coded and routine clinical specimens. Uncentrifuged cerebrospinal fluid (2 ml) was extracted under acidic conditions, derivatized, and analyzed by frequency-pulsed electron-capture gas-liquid chromatography on large-bore fused silica polar and nonpolar capillary columns. The frequency-pulsed electron-capture gas-liquid chromatography profile of carboxylic acids (C_2 through C_{22}) along with identification of tuberculostearic acid, established by retention time comparison of derivatized tuberculostearic acid derivatized sample extract, strongly suggests the presence of Mycobacterium tuberculosis in patients with lymphocytic meningitis. Results from 41 coded cases and 75 clinical cases showed that the frequency-pulsed electron-capture gas-liquid chromatography test had a specificity of 91% and a sensitivity of 95%.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL Research Publications Branch	22b TELEPHONE (Include Area Code) 202-284-1381
	22c OFFICE SYMBOL R.P.B.